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Review

Unsolved problems in biology—The state of current thinking

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A B S T R A C T

Many outstanding problems have been solved in biology and medicine for which scientists have been awarded prestigious prizes including the Nobel Prize, Lasker Award and Breakthrough Prizes in life sciences. These have been the fruits of years of basic research. From time to time, publications have appeared listing "unsolved" problems in biology. In this article, I ask the question whether it is possible to have such a list, if not a unique one, at least one that is analogous to the Millennium Prize in mathematics. My approach to finding an answer to this question was to gather views of leading biologists. I have also included my own views. Analysis of all the responses received over several years has convinced me that it is difficult, but not impossible, to have such a prize. Biology is complex and very interdisciplinary these days at times involving large numbers of teams, unlike mathematics, where Andrew Wiles spent seven years in complete isolation and secrecy solving Fermat's last theorem. Such an approach is simply not possible in biology. Still I would like to suggest that a similar prize can be established by a panel of distinguished scientists. It would be awarded to those who solved one of the listed problems in biology that warrant a verifiable solution. Despite many different opinions, I found that there is some commonality in the responses I received – I go on to discuss what these are and how they may impact future thinking.

Contents

1. Introduction

First, a few words about the Millennium Prize that was established by London Clay. It was established in 2000 by the Clay Mathematics Institute with a fund of $7 million for seven unsolved problems, $1 million going to anyone who solved any of these seven problems. For the history of the prize, see [http://www.claymath.org/millennium/]. Since then, only one problem has been solved.

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It was by the Russian mathematician, Grigori Perelman, who solved the Poincare conjecture and was awarded the prize in 2010, but refused to accept it.

There are many problems in biology that may be called "outstanding." However, it appears that this word means different things to many biologists and their responses to the question, "what are the most outstanding problems in biology," could be very different. The biologists I have interviewed over many years range from Nobel Laureates to Members of the U.S. National Academy of Sciences to Fellows of the Royal Society and biologists from leading universities who have won major awards in biology.

I first compiled a list back in 1990 (Dev, 1990) following a series of interviews with distinguished biologists. Subsequently, I discussed some of these topics in a paper (Dev, 1997) on the changing face of biology. In a later paper (Dev, 2009); I expanded on why it is essential to have meaningful conversations between physical scientists and biologists for modern biology to progress, because biology has become very interdisciplinary. Much of the pioneering work in biology, as it is practised today, would be virtually impossible without proper input from physics, chemistry, engineering etc., be it gene sequencing, high-throughput screening, 3-D imaging or, e.g., applications of nanoparticles in targeted drug delivery and gene therapy. This aspect of interdisciplinarity was further discussed (Dev, 2010), while critically analyzing new biology and the biological revolution. In 2006, and 2007, PLoS published a series of articles (Holmes, 2007; Elder and Hamilton, 2007; Mitchell, 2007; Eisen et al., 2007; Lenski et al., 2006; Wingreen and Levin, 2006) after asking several prominent biologists their views on some of the fundamental problems in biology [see List 2]. Nearly two years ago, I started sending out multiple emails to leading biologists [see the Acknowledgment section], attaching both lists and asking their opinion as to what they consider the most outstanding problems in biology. I discuss these along with my own views. It is clear that there is no uniform set of answers.

It is worth remembering that some of the problems in mathematics are more than a century old. But for the fact that Andrew Wiles solved the Fermat's last problem, conjectured in 1637 before the Clay Prize was established, he would have been, no doubt, a strong candidate for the Millennium Prize for mathematics. Readers interested in the history of this simple conjecture are referred to: http://en.wikipedia.org/wiki/Wiles'_proof_of_Fermat's_Last_Theorem (Accessed, January 12, 2015).

What matters at the end is not the amount of money but the prestige attached to a prize. In this respect, the Nobel Prize will remain paramount as well as the prestigious Lasker award http://www.laskerfoundation.org/awards/ (Accessed January 12, 2015); some of the past winners of this Lasker prize went on to win the Nobel Prize. The Nobel Prize will remain, by all measure, the most coveted prize, although some of the current prizes given for life sciences are in the millions of dollars and given to as many 10 people in the same year with a value of $3 million each https://breakthroughprize.org/ (See under life sciences, Accessed January 12, 2015). How the winners of the various prizes in life sciences are chosen is a subject of another paper [under review].

I compiled the following [List 1] from my interviews and it includes my personal opinion [1]. After the series of papers was published in PLoS [2], I revisited the topic since so much seems to have changed over the years. The lists, in no order of preference, were as follows. List 2 refers to the papers that appeared in PLoS.

1.1. List 1

1. Origin of life
2. Genetic and Molecular basis of Neural Specificity
3. Gene Regulation in Animals and Plants
4. Developmental and Behavioral Biology
5. Protein Folding and Prediction of Three-dimensional Structure from Amino Acid Sequences
6. The Problems of Evolution

1.2. List 2

This is all from PLoS Biology following a challenge. These appeared in 2006 and 2007 (References above), and also as a collection in the following website: http://www.ploscollections.org/article/browse/issue/info%3Adoi%2F10.1371%2Fissue.pcol.v. 06.i01 (Accessed January 12, 2015).

7. Viral Evolution in the Genomic Age
8. Stoichiometry and the New Biology: The Future Is Now
9. The Genetics of Brain Wiring: From Molecule to Mind
10. Environmental Shotgun Sequencing: Its Potential and Challenges for Studying the Hidden World of Microbes
11. Evolution, Interactions, and Biological Networks
12. Balancing Robustness and Evolvability
13. Cooperation among Microorganisms

There is little in common between the two lists. More often than not, it appears to be the opinion of the individual scientist, although I did receive some common answers. It would practically be impossible to cover all the items that appear on these two lists. My intention here is to discuss the viewpoints of some of the leading scientists, along with my own views, on this very important topic. First, I describe some of the major prizes in the life sciences. Some of these prizes are given only for specific branches of biology such as neuroscience, basic medical research, the environment, etc.

I want to emphasize that this paper does not deal with some major problems like environment, climate change and energy, nor does it deal with "developmental and behavioral biology," except a brief mention on the section of neural plasticity. These, in my opinion, to quote Weinberg, a scientist I interviewed, "deserves its own stand-alone status." I have not sought opinions from historians, sociologists or philosophers of science. The views expressed here are not the results of a general survey; instead they are the opinions of some internationally known biologists who have advanced basic knowledge in many ways that have also found relevant applications. A legitimate question may arise why did I concentrate on the so-called "establishment scientists." This is partly because I have been motivated by the speeches delivered at the Lindau conference by as many as 37 Nobel Laureates who discuss almost every aspects of many fundamental biological problems from aging to cancer to evolution etc. These scientists interact with as many 600 young scientists selected from round the world. The readers may be interested in the 2014 Lindau meeting in physiology and medicine http://www.nature.com/lindau/2014/index.html (Accessed January 12, 2015) http://www.mediatheque.lindau- nobel.org/videos/lectures#page=1&sort=4&opt=desc&pagesize=1&f=1&sci=212 (Accessed January 12, 2015) http://www.lindau nobel.org/2014_Lindau_Meeting Physiology_Medicine.AxCMS?ActiveID=2782 (Accessed January 12, 2015).

2. Biological sciences discovery

Under this heading, I discuss some of the major prizes that are awarded for achievements in the life sciences. Since the topics relate to unsolved problems of biology, I will mention some of the major discoveries of the last 50 years. CASW, the Council for the Advancement of Science Writing, has catalogued fifty major discoveries from 1960 to 2010. http://casw.org/casw/article/50-science-sagas-50-years (Accessed January 12, 2015). These are:

(1) Translation of genetic code within the cell,
(2) Recombinant DNA which has revolutionized production of many important drugs,
(3) Eradication of Smallpox,
(4) Identification of HIV,
(5) PCR, Polymerase Chain Reaction, a technique to amplify DNA from microscopic amounts of materials that may be available,
(6) Human embryonic stem cells including IPS (Induced Pluripotent Stem Cells) which allows human skin cells to be reprogrammed into embryo-like stem cells,
(7) Decoding of human genome although the announcement at the time showed gaps in the map. There have been major advances, especially in sequencing, since that announcement.

Among many of the responses I received, a large majority mentioned several aspects of neuroscience. This is not surprising since the brain remains the most uncharted area in humans. A list of unsolved problems in neuroscience can be found in http://en.wikipedia.org/wiki/List_of_unsolved_problems_in_neuroscience (Accessed January 12, 2015).

For the sake of compactness, I discuss only two major awards, one being the Lasker Awards, established in 1945, and the recently instituted Life Sciences Breakthrough Prize funded by major organizations such as Google, Apple and Facebook. Lasker has been known as "the America's Nobel" since a number of scientists who won the Lasker award went on to win the Nobel Prize. To quote from the Lasker website http://www.laskerfoundation.org/awards/ (Accessed January 12, 2015). Lasker awards recognize "the contributions of scientists, physicians, and public servants who have made major advances in the understanding, diagnosis, treatment, cure, and the prevention of human disease." Some of the winners of this prestigious award have been Mario Capecchi, Martin Evans, Oliver Smithes, Randy Schekman, James Rothman and Sidney Brenner, to name only a few, who all went on to win the Nobel Prize.

Breakthrough Prize is not as well established as others. This is not to underestimate the achievements of the winners, some of whom I had corresponded with. Apart from Shinya Yamanaka, a Nobel Laureate, the list includes such leading biologists as Robert Weinberg, Eric Lander and Bert Vogelstein. A third is the Copley medal, awarded by the Royal Society, which includes winners like Paul Nurse, a Nobelist for his elucidation of the control of cell division and the fourth is the E B Wilson Medal by the American Society of Cell Biology to Osamu Shinomura, Martin Chalfie and Roger Tsien.

3. How were the interviews conducted?

The responses I received were compiled mostly by correspondence through emails but some of them were conducted by telephone calls and face-to-face interviews. Occasionally, I sent the scientists excerpts of my interviews and, in one case, the full transcript. For this particular article, I started by sending the scientists the two lists I prepared, the first one [List 1] is taken from my 1990 paper which dealt, principally, with the migration of physical scientists who transitioned to biology and went on to make great contributions to biology. This list also included some biologists who did not start out majoring in physical sciences but had interactions with leading scientists such as Fermi, Curie and Delbruck. Since my publication (Dev, 1990) I have been tracking the field, what I call outstanding/unsolved problems in biology.

To start with, I sent out Lists 1 and 2, with the following comments, "There is not much in common between the two lists. Clearly, more often than not, it appears to be the opinion of individual scientist, although I did have some common answers. It would practically be impossible to cover all the items that appear on these two lists but your own views, as to what are the most fundamental problems in biology and medicine, would be greatly appreciated. Some of the responses were short and some quite long. Whenever possible, I will quote them ad verbatim.

4. Responses to outstanding problems in biology

Venki Ramakrishnan (Cambridge University). He won the 2009 Nobel Prize for "studies of the structure and function of the ribosome". His response was the shortest that I received. He simply said "*It seems to me that nearly all of the topics from List 2 are details or subsets of the much broader questions of List 1, which seems fairly comprehensive to me.*" Although it is encouraging to read his comments since I compiled the List 1 many years ago, which, I believe, is still very relevant.

Mario Capecchi (University of Utah), currently Distinguished Professor of Human Genetics and a co-winner of the 2007 Nobel Prize in Physiology and Medicine for discovering a method to create 'knock-out' mice by turning off specific genes. His response was short but very perceptive. He said, "I would agree that our most glaring ignorance is how our brain works which can be couched in various paradigms" but he also mentioned that a problem that interests him, "i*s the genetics of biological* innovation. *That is, how a common set of genes is used to create an entirely new function."*

Professor Robert Weinberg (MIT), winner of many prestigious prizes, and is internationally known for his discovery of the first oncogene, Ras, and the isolation of first tumor suppressor gene, Rb. He is a founding member of the Whitehead Institute for Biomedical Research. His response was as follows: "*I must say that, just as you yourself have said, the choice of these topics seems very idiosyncratic — a diagnostic bias depending on whom you have asked. Some are quite idiosyncratic — origin of life is not something people work on that much because it's so far away from resolution. Gene regulation is something that is being rapidly solved and does not represent a major conceptual problem. Developmental and behavioral biology are two major topics, each of which is surely a major problem and each of which deserves its own stand-alone status. I would think that the problem of brain function continues to be of transcending importance and am not sure how it relates to* "*behavioral biology*". *Protein folding is something that is gradually being solved and is not really so intractable, given the use of existing structures to predict, by homologies, new ones. The problems of evolution continue to be major and I would buy into them. List 2 is, if anything, even more idiosyncratic and reflective of certain people's biases. Viral evolution is not a major conceptual problem and is being rapidly worked out in terms of sequencing viral genomes etc.* "*Stoichiometry…*" *is obscure. I think that a major unsolved problem that has ramifications on multiple fields is the following: How do signal-processing proteins within cells create the complex circuitries that determine cell behavior* (*specifically an understanding of how these biological* "*integrated circuits*" *operate*)? *We are still many years away from solving this problem, and it lies at the heart of understanding developmental biology, cancer pathogenesis. The other topics in List 2 seem, very idiosyncratic!*".

Professor Philip Sharp (MIT). Sharp shared the Nobel Prize with Richard Roberts in 1993 for the discovery of RNA splicing. Earlier, I discussed with him about the doubt some scientists have expressed on the central theme of molecular biology [3]. His comments for the current topic were as follows: "*The origin of life and its evolution is clearly the central problem in Biology. The most challenging central questions that will be investigated for decades,* *perhaps centuries, is an integrated model of the processes constituting and maintaining in a dynamic fashion the state of the cell. In many programs this is called* "*Systems Biology*."

The questions that arises is whether even such an integrated approach as systems biology would be able to answer in a unified way the pertinent questions that Sharp and Hood have raised (Dev, 2009).

Professor Lynn Enquist, Neuroscience Institute (Princeton University). Enquist's lab at Princeton works on neurovirology and, in particular focuses on the mechanism of herpesvirus pathogenesis. Since he is a neuroscientist, it is not surprising that his responses are related to his own specialty. He wrote: "*The major and exciting problem is the brain – how does it work? How does the constellation of cells in this amazing tissue sense the world and make sense of it (and enable us to respond). Everything that we do, think, and respond to is the result of actions of single cells. How do the molecular and cell biology that we understand so well become so integrated to produce behavior, dreams, aspirations, etc*."

Professor Howard Berg (Harvard University). I first interviewed Berg for my 1990 paper (Dev, 1990) where some excerpts are given. Berg is a world authority on bacterial flagellar motion. He was the first one to construct a 3-D Tracking Microscope to study bacterial motility. He made a grand sweep on the points I raised in my Lists 1 and 2. They were very interesting comments, to say the least, which were as follows: "*I am not very good at broad generalizations, or fond of medical applications. I work, as a basic scientist, propelled by curiosity, on the molecular biology of behavior, which relates to items 2, 4 and 9 of your List 1 and 2 (all important), but at the single-cell level. E. coli has a single-celled nervous system. It has taught us about nanotechnology, e.g., molecular machines, such as receptor clusters, a signaling kinase, and the flagellar rotary motor. It has taught us about signaling networks. How do cells sense and respond to changes in their environment?*"

He also wrote: "*Items 3, 5 and 6 of list 1 are all profound, item 1 is less so, because facts are few. Perhaps one should work, instead, on life on other planets. There are planets everywhere, and presumably life almost everywhere. But it is unlikely that we will be able to look beyond our own solar system. I am certain that intelligent life exists or has existed in other solar systems, but the chance that our technical prowess and theirs peaks in the same era seems remote. Suppose we had dinosaurs who knew about telecommunication? Alas, we would be 65 million years or more out of sync. Will our successors, 65 million years from now, discover old radio sets?*

List 2 seems to me too topical except for item 9 on list 2. Topics to be added might include climate change (or, more generally, the environment) and what we might be able to do about it. One of the problems of evolution is that evolution ceases, for a given species, when that species goes extinct.

If you want a really long-term view, when the sun runs out of hydrogen, it will expand into a red giant and the earth will be incinerated. But that's in another 2 billion years. Will we have moved by then? The experiment of life on Earth is already 2/3rds over."

Professor Immo Scheffler (UCSD) is an expert on mitochondrial biology with a very popular textbook on the subject. Although he is currently an Emeritus Professor, he is as active as ever! Over the years, I have had many conversations with him. Regarding his views on what are the outstanding problems in biology, his comments are very interesting. Like Professor Berg, he does not think that a simple list is "sufficient." He does distinguish the "problems that are almost purely of theoretical and intellectual importance:" His detailed response was as follows: "*(1) The Brain: evolution/development, learning, memory, creativity, behavior, abnormalities; (2) Metabolism. Here I mean not only having a metabolic chart with all the possible reactions and intermediates, but a complete quantitative understanding (networks, systems biology, flux control) to deal with nutrition, drugs, genetic and environmental perturbations, and ultimately aging. I am not in favor of life-span extensions as fantasized by a few individuals; (3) Gene Regulation: all along we have not been humble enough to appreciate the complexity of this problem. The recent developments in epigenetics, micro RNAs, macro RNAs, nano RNAs etc. are far from understood. Under this heading one can include embryonic development; (4) I am fascinated by the recent emphasis on the fact that each human lives in symbiosis with ~1–2 kg of bacteria; their potential significance is just being glimpsed; (5) Microbiology: large numbers of pathogens threaten us and our food supply (see Science 337: 636–638 (2012)); (6) Macrobiology, Environmental Studies, Conservation Biology." He also made brief comments on population control, clean and abundant energy, which he considers "important problems" that "are practical and political.*"

Professor Krishnaswamy Vijayraghaban, Distinguished Professor and Director of the National Centre of Biological Sciences, Bangalore, India. According to him, the major problems to be solved are "*(1) Visualization of the dynamics of localization and function of individual molecules in living cells and tissues. Till recently, our views of cellular function came from mere snapshots. This has changed recently, but an ability to view several molecules in cellular signaling pathways in live cells as they interact with other molecules is becoming possible and will reveal new ways of biological function.*

(2) Deciphering the principles underlying long-range interactions of cells with each other and with the environment. Groups of cells function in coordinated ways displaying properties distinct from individual components of the tissue they make up. How forces, chemical signals and history of exposure to various cues shape tissue properties will reveal new principles through the use of the tools of soft-matter physics in biology. (3) Examining the dynamic properties of neurons in defined networks, relating this to physiological outputs of the network and to behavior to decipher the rules that specify the development, emergence and maintenance and function in the brain".

Apart from the names I have mentioned above, I have also talked with many other leading biologists. In particular, I mention the name of Professor Leroy Hood [Head of the Institute for the Systems Biology, Seattle] on systems biology. He gave me not only a long telephone interview, but also sent me pdf files of some of his articles. I also had many conversations with Veronica Shubayev, *(UCSD)*, an Associate Professor, Department of Anesthesiology. Her primary interests are to identify the "mechanism of neuronal damage" and "to elucidate the role of MMPs in survival." As for origin of life, she felt "there are profound philosophical and scientific gaps in our understanding of the origin of life, which need be filled."

Since I completed the interviews, a series of articles recently appeared as Open Questions, BMC Biology (Charles et al., 2014; McConville, 2014; Sommer, 2014; Germain, 2014; Magurran, 2013; Stockinger, 2013; Adams, 2013; Petsko, 2013; Cole, 2013; Hurst, 2013). A topic like cellular and tumor heterogeneity (Sommer, 2014), important though it is, can be considered as subsets of what has already been discussed. The fact that primary tumors can be different from metastatic ones in the same person is well known.

There is no doubt that the kind of questions Lukas Sommer has raised (Sommer, 2014) will be very helpful in personalized medicine but the overall program still comes under the umbrella of genome organization, where many basic problems need to be solved. Similarly, the question as to how far genomics has not come (Adams, 2013), there is no doubt that we still have a way to go but what has been achieved, since the announcement of first rough map of the human genome, has been extraordinary. Similar comments can be made with respect to the genome organization. As for genomics, its importance can be gauged by the fact that the WHO has declared grand challenges namely, "what are the major Grand

Challenges and bottlenecks to be overcome for genomics to be harnessed and used by developing countries to address the greatest public health problems over the next 10 years?" http://www.who.int/rpc/grand_challenges.pdf (Accessed January 12, 2015.)

5. Comments on the responses received

Origin of life and its evolution: It can be easily seen from the responses that there are, at times, diametrically opposing views among leading biologists. Take, for example the following, Weinberg says, "*Origin of life is not something people work on that much because it's so far away from resolution." He also says, "List 2 is, if anything, even more idiosyncratic and reflective of certain people's biases." Berg also makes similar remarks, writing "(3), (5), and (6) of list 1 are all profound. (1) is less so, because facts are few. Perhaps one should work, instead, on life on other planets.*" Sharp, on the other hand says, *"The origin of life and its evolution is clearly the central problem in Biology.*"

On the first thought, I agreed with Weinberg and Berg. Evolution is too vast a subject and, literally, thousands of papers have appeared on the topic. I have discussed this in my previous paper (Dev, 2010) under the heading, "A full understanding of evolution still needs new input." In fact, Howard Berg's idea is appealing enough for NASA to fund a project with such a theme. The Jet Propulsion Lab (JPL), part of California Institute of Technology, put out a press statement with the headline, "How Did Earth's Primitive Chemistry Get Kick Started?" http://www.jpl.nasa.gov/news/news.php?release=2013-235 (Accessed January 12, 2015) In fact, a large variety of approaches have been taken to account for the origin of life — Astrobiology (Benner, 2010), Information theory (Kuppers, 1990), Role of RNA (Gilbert, 1986), A statistical model (Dyson, 1982), Role of quantum mechanics (Davies, 2004), and also the idea first propagated by Russell that life originated from hydrothermal sources (Russell and Hall, 1997). Recently, it has been suggested in a controversial paper (Sharov and Gordon, 2013) that the origin of life can be extrapolated similar to Moore's law that number of components per chip would double every 12 months. Extrapolated back that gives the age of earth over 9.5 billion years, a much higher figure than the conventionally accepted figure of just over 4 billion years. This article has been well summarized in Technology Review that life began before earth.http://www.technologyreview.com/view/513781/moores-law-and-the-origin-of-life/ (Accessed January 12, 2015). For the original paper, rather than the review referred above, please see Sharov and Gordon (2013). A timeline of human evolution starting 55 Million Years Ago (MYA) when the "first primitive primates evolved, lived in the shadow of the dinosaurs" right down to "4000 to 3500 BC — the Sumerians of Mesopotamia developed the world's first civilization" http://www.newscientist.com/article/dn9990-introduction-human- evolution.html?full=true#.U5cGOvidWpQ (Accessed January 12, 2015).

In an earlier publication (Dev, 2009), I discussed how several aspects of *evo-devo* (evolution-development), a new branch of biology, have confirmed Darwin's inspired guesses as to when it was that specific genes during embryonic development of the Galapagos finches decided the size and shape of their beaks.

However, all these examples I have given show how complex and challenging the problem of origin of life is and, although this may never be solved, it is tempting enough to be one of the major unsolved problems in biology. In a special issue in September 2014 issue of Scientific American (Vol. 311, No. 3, Sept. 2014), nine articles have been published dealing with several aspects of "The Human Saga," starting with "Evolution rewritten" to "Still evolving (After all these years)." These clearly establish that there are many challenges left and the final word has not yet been spoken. In a leading article on "Welcome to the family," in this special issue, Bernard Wood says, "The latest molecular analyses and fossil finds suggest that the story of human evolution is far more complex … than anyone imagined."

Systems Biology: I could not agree more with Sharp who said, "The most challenging central questions that will be investigated for decades, perhaps centuries, is an integrated model of the processes constituting and maintaining in a dynamic fashion the state of the cell. In many programs this is called "Systems Biology." I discussed in detail both systems and synthetic biology in an earlier publication (Dev, 2010). In particular, I discussed the contributions of Noble in systems biology. I also emphasized the work of the Institute for Systems Biology in Seattle directed by Hood and the Bauer Institute at Harvard. Systems biology needs, to quote Leroy Hood, "*a cross-disciplinary environment composed of biologists, chemists, computer scientists, engineers, mathematicians, physicists, and physicians speaking common discipline languages.*" The Koch Institute for Integrative Cancer Research at MIT takes a similar approach to systems biology and "co-localizes" faculty members from the departments of Biology and Engineering and also many members of the Whitehead and Broad Institutes. Such an integrated approach has been a key ingredient of the rapid advances, rather than projects being tackled separately by different departments across the campus.

A recent review lays out the scenario of systems biology from biological network to modern therapeutics (Somvanashi and Venkatesh, 2014). The authors start with the assumption that a disease diagnosis is similar to a fault diagnosis in an engineering system and apply engineering methodologies to human disease. They go on to show a synthetic diagram of information processing in a cellular network and, more impressively, a systemic map of insulin resistance and defective metabolic defective homeostasis. The ultimate idea here is to design drugs and, hopefully, appropriate therapeutics, based on such network connectivity. How can such a network be rewired has been discussed (Fintoft, 2004).

Darwin's theory of evolution is now well established. Weitz et al (2007) in an interesting article discusses the role of biological networks and their belief "that the lens of evolution provides an exciting opportunity to link disciplines in ways that address fundamental challenges in biology.

It is very important to point out the classic work done by Noble on systems biology. He has nicely reviewed the subject starting with the work of Hodgkin and Huxley. He challenges the "*need to develop the theoretical framework required to deal with multilevel interactions*" (Noble, 2010). As one might have noticed the network connectivity diagram is far from simple. My personal approach is one of integration like that of Leroy Hood at the Institute for Systems Biology (ISB) where both theory and experiment go hand-in-hand. Hood terms it "P4 Medicine." He says, "P4 medicine that is predictive, preventive, personalized, and participatory." Hood takes a cross-disciplinary approach involving biology, chemistry, computer science, engineering, mathematics and physics. He calls it a "Holy Grail." Using this kind of strategy, Hood and his team have been able to identify, e.g., "new protein modification critical to growth of TB pathogen."

Four emerging applications of system biology have been discussed in a review (Chuang et al., 2010), these being: (*a*) pathway-based biomarkers, (*b*) global genetic interaction maps, (*c*) systems approaches to identify disease genes, and (*d*) stem cell systems biology. A meta-analysis of systems-biology publications over the past decade has been done from 2001 to 2009.

Let me illustrate with just one example. These days we talk about "Big Data." The question remains how Bioinformatics and Systems Biology can be brought together to derive most relevant therapeutic information from a vast amount of omic data obtained from DNA, RNA, protein and metabolomics from a "single biopsy sample" in a reasonable length of time to be useful. This has been discussed (Prahlad et al., 2012).

Neural plasticity. The Oxford English Dictionary definition of plasticity, when applied to biology is, "The adaptability of an organism to changes in its environment or differences between its various habitats." And, the brain is exactly that. It is malleable. It is a generalized term and it can be both synaptic and non-synaptic. Changes can arise from changes in behavior, environment, neuronal changes or results from simple bodily injury. Work on the brain is very fundamental for most biologists. It covers such a wide gamut of topics as discussed before, from neural plasticity to memory to behavior. A good description of neuroplasticity can be found in http://en.wikipedia.org/wiki/Neuroplasticity. In particular, a review on neural plasticity in the aging brain appears in Burke et al (2006). A multi-chapter book on neural plasticity by many leading biologists covering genetics, molecular, behavioral and brain imaging techniques appears in Bermidez-Rattoni (2007). Although much work remains to be done, a remarkable paper has recently appeared (Grosse-Wentrup et al., 2011) which demonstrates how, in principle, neural plasticity can be induced using a brain-computer interface to restore function, eventually, as a therapeutic application. The article goes on to discuss future challenges.

A recent proposal to collaborate, between the US and the European effort is a testimony to this. The US project, funded by a $1 billion program, called BRAIN (Brain Research through Advancing Innovative Neurotechnologies), and the European Union $1.3 billion dollars program is known as HBP (Human Brain Project). As reported by Sara Reardonhttp://www.nature.com/news/brain-mapping-projects-to join-forces-1.14671, "the BRAIN initiative aims to create tools for imaging and controlling brain activity, while the HBP seeks to create a working computational model of the entire brain." This will be essential in order to answer questions raised by many biologists. Future research, according to a Stanford University website, aims to "develop lifestyle behaviors and medications that could improve normal brain development as well as repair damaged brains." http://www.stanford.edu/group/hopes/cgi-bin/wordpress/2010/06/neuroplasticity/ (Accessed January 12, 2015) In an article in Discover magazine by one of the leading neuroscientists in the world, David Eagleman beautifully summed up what are the important problems yet to be solved about the brain (Eagleman, 2007). These are (I quote ad verbatim): "*How is information coded in neural activity? How are memories stored and retrieved? What does the baseline activity in the brain represent? How do brains simulate the future? What are emotions? What is intelligence? How is time represented in the brain? Why do brains sleep and dream? How do specialized systems of the brain integrate with one another? And, finally, What is consciousness?*" He goes on to say that even if we could answer these questions partially, "it could restructure our understanding," a statement which can be hardly overstated.

Although the joint US and European collaborative program on the brain initiative has been criticized because of the vast expenses involved and doubt expressed as to how much such projects would enhance our understanding, my argument is that similar doubts were voiced when the sequencing of the human genome was proposed. Now, after several years we find that the cost has gone down from $2 billion to almost $1000 for sequencing the whole genome of an individual. Far more important is the fact that such an effort is paying off handsomely in many different areas in terms of basic understanding as well as in its applications.

Gene regulation in animals and plants. Berg, in my opinion, correctly says that this is "profound." This is supported by Scheffler, "*Gene Regulation: all along we have not been humble enough to appreciate the complexity of this problem. The recent developments in epigenetics, micro RNAs, macro RNAs, nano RNAs etc. are far from understood. Under this heading, one can include embryonic development.*" A classic paper on gene regulation by Ptashne (Ptashne, 1986) shows that "transcription of genes can be controlled both by regulatory proteins that bind to sites on the DNA either nearby or at a considerable distance." Recent research has clearly established the important role of epigenetics mentioned by Scheffler. One good example is that of identical twins. Previously, it was thought that phenotypic differences in such twins were due to environment but new results—both theoretical an experimental-have clearly established that epigenetics can account for such differences (Wong et al., 2005). Evidence has been provided that animals ingesting plant miRNA can regulate their gene expression (Vaucheret and Yves Chupeau, 2012). The important role of the non-coding microRNA has been established in worms, flies, plants and mammals. This has been reviewed and summarized by He et al (2004). It is relevant to mention here that all major discoveries in eukaryotes were first found in plants. In a recent editorial on "Genomes gone wild" in *The Scientist* (Scudellari, 2014) magazine, Scudellari says, "weird and wonderful, plant DNA is challenging preconceptions about the evolution of life, including our own species".

To the list mentioned above, I would personally like to add the following points:

Stem cell and regenerative medicine. Despite recent retraction of some papers from *Nature* by scientists from the Riken Institute and Harvard University, its potential remains formidable. This is especially true after Yamanakawa's path-breaking work on induced pluripotent stem cells (iPSC) from adult skin cells by using only four transcription factors for which he was awarded the Nobel Prize. A large number of projects are currently being pursued on stem cells, far too numerous to deal with here. Suffice it to say that the efforts range from cure of Alzheimer's disease to cancer and many other diseases. Billions of dollars have been spent since President Nixon's declaration on the 'war on cancer' but we are nowhere near to fulfilling that dream, although progress has been made. Many articles have been written on the progress we have made for some of the cancers. I would like to mention, in particular, the interview at the Lindau meeting with Michael Bishop who shared the Nobel Prize, along with Harold Varmus "for their discovery of the cellular origin of retroviral oncogenes" http://www.nature.com/lindau/2014/index.html (Accessed January 12, 2015). Recurrence of cancer has been attributed to the inability of killing cancer stem cells, which are few and far between, by any of the conventional methods available, namely, chemotherapy, radiation, surgery, etc. The challenges involved have been adequately described by Blanpain (2013). A recent comprehensive review (Tabar and Studer, 2014) on the use of human pluripotent stem cells [hPSC] for regenerative medicine shows that it is now possible to derive disease-relevant cell types from such cells and also describes the challenges that remain before their full implementation in clinical settings.

It is unfortunate, and this occurs especially in life sciences, that when it comes to applications of truly groundbreaking discoveries, media hype creates unjustified optimism. A case in point is small interfering RNA (siRNA), a serendipitous discovery that allows a break in the information flow from DNA to RNA to protein and silences the bad genes. Its mechanism could not be explained for years but, eventually, Fire and Mello were awarded the Nobel Prize for it in 2006. The phenomenon of gene silencing was discovered long before 2006 in plants but it was the work of Fire and Mello that proved that such silencing occurred also in eukaryotes. However, its practical applications in medicine, for example, have hit many hurdles (Dev, 2005). I mentioned briefly the role of microRNA (miRNA) there. However, this topic has assumed major importance as can be seen in a 2008 review by Baltimore et al. (2008). It shows that miRNAs affect mammalian immune system. Therefore, far more basic work is necessary before research in animal models can be effectively applied to humans.

Biologicial Aging: Kumlin, in a paper on theories of aging (Jin, 2010), asks the following questions: "Why do we age? When do we start aging? What is the aging marker? Is there a limit to how old we can grow?" These questions have existed in one form or another for hundreds of years, but there has never been a satisfactory answer. As Scheffler pointed out in his response, aging is an important phenomenon. There is a common misunderstanding in some media that research in aging means extending life. Life extension, as understood in ordinary parlance, is meaningless if a person lives longer, but is riddled with disease. The whole idea here is to be able to answer the kind of questions that Lin has asked. Hayflick's limit on cell senescence and the role of the Sirtuin gene have been extensively dealt with.

A beautiful power-point presentation from NTNU and St. Olavs Hospital in Trondheim, Norway, on "Biological aging-theories and research" states, quite rightly, in my opinion, that "aging is a central aspect of human biology … and one of the least understood." Biological ageing can be at different levels that involve DNA, organelles and cells, tissues, organs etc. A timeline on Life Perspective shows the approximate duration starting with Fertilization –> Fetus (0.7–0.8 yrs) –> Birth –> Infant (0–3) –> Adolescent (onset-10–20) –> Parent (onset-max. Fertility-16–22) –> Middle age (decreased fertility: for women 40–50 yrs and for men after 50) –> Healthy Old age (duration 0–30) –> Frailty (duration 0–5) –> Death http://kavlisenter.no/admin/uploads/Sletvold.pdf. It must be understood that individuals can age at very different rates depending on various factors that may include, "species, gender, relative brain weight/brain mass (BM), length of growth periods, body temperature, inheritance, nutrition and environmental conditions." It simply shows that a large number of variables need to be taken into account before a unified theory can be established that is verifiable.

Many theories on ageing have been proposed but none has been adequate to explain the various questions that I have catalogued. These include: dependence of life span and how good is the DNA-repair rate; mitochondrial theory; protein accumulation theory; network theories, and telomere loss etc. http://kavlisenter.no/admin/uploads/Sletvold.pdf.

Recently, a few world-renowned geneticists, such as David Botstein (Princeton) and Cynthia Kenyon (UCSF) have been employed by Google to work on aging. Kenyon has shown that mutation of a single gene allows a roundworm to live twice as long without such manipulation. However, when she was asked in an interview, how long would it take for such a pill to be available for human beings, she said, "It takes very long, to find out if the same molecular mechanism, which influences aging in the tiny round worm, can also be applied to higher organism." http://www.nar.uni-heidelberg.de/en/service/int_kenyon.html (Accessed January 12, 2015).

The discussion on biological aging—theories and research [above] clearly lays out why aging is such a fundamental problem along with the others mentioned.

There have been many fundamental discoveries in biology and, quite justifiably, the scientists have been awarded many prestigious prizes. The scientists involved in such work were not necessarily motivated by such prizes but driven by curiosity. It goes without saying that many of these discoveries that answered the ultimate questions raised and, in the process, also changed many human lives, would have easily come under the category of unsolved problems, had there been such a list like the Millennium Prize in mathematics. However, there will continue to be many more unsolved problems and new challenges in the future. I would give one example, namely, the discovery of DNA cloning and genetic engineering by Herb Boyer and Stanley Cohen resulting in the 2004 Shaw Prize for life, http://www.shawprize.org/en/shaw.php?tmp=3&twoid=65 (Accessed January 12, 2015).

6. Conclusions

This article is an attempt to list some of the unsolved problems in biology by seeking opinions of many leading biologists, along with some of my personal views. As can be seen, the opinions can be very different on the same topic. Yet, one can find some commonality in the responses. In the recent past, at the invitation of some editors of leading biology journals, similar articles have been published. Although biology has become complex and interdisciplinary, I am of the opinion that it would be possible for a panel of distinguished biologists to draw up a list of such unsolved problems, which would be similar to that in mathematics where seven problems have been listed for the Millennium Prize and only one has been solved. The final proposed list is: (1) Origin of life and evolution, (2) Systems biology, (3) Neural plasticity, (4) Stem cell and regenerative medicine, (5) Gene regulation in animals and plants, and (6) Biological ageing.

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